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THE EFFECTS ON STREPTOCOCCI OF SERA OF COLD-BLOODED ANIMALS.*

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THE bacteriological investigations of scarlet fever have shown that streptococci practically always are associated with many of the severe complications of this disease, and that these organisms can be isolated from the internal organs and bone marrow of a large percentage of the fatal cases. These organisms can also be isolated from the blood, during life, in a small percentage of cases, as was shown by Hektoen, Lemoine, Slawyk, and others. Although it cannot be claimed that these investigations have shown that scarlatina is a streptococcus disease, there can be no doubt but that the complications and mortality of this disease would be greatly reduced if we were able to cope successfully with the secondary streptococcus infections.

Exhaustive studies of streptococcus infections are especially indicated in view of the fact that at present but little is definitely known about the finer mechanisms of these infections. Normal human serum is strongly bactericidal, *in vitro*, for some pathogenic bacteria, as B. typhosus and B. dysenteriæ. The all-important question here is: What brings about the conditions which permit these organisms to multiply in the body and produce their respective disease? Streptococci, on the other hand, multiply very rapidly in normal human serum and in scarlatinal serum, as was shown by Dr. Weaver and myself.[1] This would seem to indicate that the body finds no protection against streptococci in the serum alone, but that the struggle between the body and these organisms must be looked for elsewhere than in the blood serum. Denys and LeClef,[2] Bordet,[3] and Marchand[4] have

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[1] *Trans. Chicago Path. Soc.*, 1903, 5, 285; *Medicine*, July, 1903.

[2] *La Cellule*, 1895, 11, 177.

[3] *Ann. de l'Institut Pasteur*, 1897, 11, 177.

[4] *Arch. de Méd. expér.*, 1898, 10, 253.

endeavored to show that this struggle falls entirely upon the phagocytes—a view that has not been very widely accepted, although it has many points in its favor. In other diseases, as for instance, diphtheria, antitoxins can be demonstrated in the blood serum of convalescents, but according to Neufeld,[5] no such antibodies can be detected in the serum of convalescents from streptococcus infections. And the investigations of Dr. Weaver appear to show that as yet but little faith can be placed upon the significance of the agglutinative reactions of streptococci with the serum of scarlatinal and other patients.

One of the most interesting developments in the recent study of streptococci is the discovery by Besredka of streptocolysin, a special hemolysin produced by virulent streptococci when grown in heated rabbit serum. I have shown that this lysin is composed of a haptophore and a toxophore group, which are firmly linked together.[6] The haptophore group may be neutralized with chicken serum, and the toxophore group can be destroyed with traces of zinc chloride or zinc sulphate. The filtrates of virulent streptococci in heated serum were found to be toxic for rabbits. The organs (especially the spleen) of a rabbit which has died from the effects of an injection with these filtrates give a strong reaction for hemosiderin, which would indicate that hemolysis has taken place during life. These results suggest that further insight into the pathogenic action of streptococci may be obtained by the minute study of the substances they produce.

Since human serum and the sera of most warm-blooded animals are not bacteriolytic for streptococci, it would be interesting to determine whether there are any sera among the cold-blooded animals which are destructive for these organisms. This work was undertaken at Wood's Hole with that object in view, but also bearing in mind the possibility of producing in some of these animals an anti-streptococcic serum of greater therapeutic value than are those so far obtained from the horse.

The sera of twenty species were tested on several strains of streptococci, by the usual plate method. The blood was obtained directly from the heart by means of a sterile pipette, allowed to clot, and the clear serum which exuded was used within three to five hours after killing the animal. One c.c. of serum was introduced into each of several small tubes, inoculated with streptococci, and glucose agar plates made at intervals. The tubes were kept in the incubator at 35° C. The counts of the colonies, which were made in about thirty hours, showed that the streptococci multiplied rapidly in most of the sera, as is shown by Table I.

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[6] *J. Am. Med. Ass.*, 1903, 41, 962.

[5] *Deutsche med. Wchnschr.*, 1897, 23, 162.

TABLE I.

The Growth of Streptococci in the Sera of Various Cold-Blooded Animals.

Streptococci	Sera	Immediately	Two Hours	Five Hours	Twenty-Four Hours
270	Butterfish	500	1,500	10,000	30,000
244	Dogfish	2,400	2,500	3,500	2,500
270	Conger eel	2,500	......	5,000	10,000
244	Flounder	100	500	1,900	5,000
270	Mackerel	1,300	1,300	5,000	∞[1]
244	Butterfly ray	4,200	......	5,000	∞
B104	Sting ray	5,000	......	5,000	∞
244	Sand shark	1,400	......	6,000	10,000
270	Dusky shark	150	......	2,000	6,000
244	Scup	600	1,200	800	8.000
244	Common skate	2,000	2,400	4,500	10,000
270	Squetrague	1,000	2,000	8,000	∞
270	Common squid	200	......	1,000	∞
270	Sturgeon	400	900	4,000	15,000
270	Lobster	3,000	5,000	10,000	3,000
244	King crab	13,000	......	19,000	800
244	Spider crab	900	......	10,000	∞

Sources of streptococci: No. 244 from blood, during life, in puerperal sepsis; No. 270 from blood during life, in scarlet fever; No. B104 from an abscess in a guinea pig which had been injected with a fungus.

The sera of several species of turtles, however, showed a very different behavior toward the streptococci than did the sera of the other animals. In these sera the organisms did not multiply, but their number decreased very markedly during the first five hours, as shown by Table II. In no instance was I able to get entirely sterile plates in twenty-four hours, although in several experiments the number of colonies dropped from several thousand to less than one hundred. Control tubes were always made, and the results noted only when the controls remained sterile. There was one strain of streptococci (No. 244) which multiplied in all sera, although less rapidly in those of turtles than in those of the other animals. If the serum was heated to 54° C., there was a rapid multiplication of all the organisms from the start. Heated serum can be reactivated by adding 0.2 c.c. of normal serum to 1 c.c. of heated serum. This does not appear to be a true reactivation, however, because nearly the same effect can be obtained by using bouillon instead of heated serum, as shown by Table II.

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[1] 8 stands for very many.

TABLE II.

The Growth of Streptococci in the Sera of Turtles.

Streptococci	Sera	Immediately	Five Hours	Twenty-Four Hours
B104	Speckled turtle	2,400	450	1,000
270	Speckled turtle	2,700	900	1,500
244	Speckled turtle	2,500	3,000	4,000
B104	Speckled turtle (heated)	1,800	15,000	∞
300	Painted turtle	3,400	480	1,800
B104	Painted turtle	2,000	400	1,200
244	Painted turtle	6,000	10,000	10,000
300	Painted turtle (heated)	2,500	∞	∞
B104	Yellow-bellied terrapin	9,000	3.000	480
270	Yellow-bellied terrapin	5,000	1,900	560
244	Yellow-bellied terrapin	2,300	4,500	2,800
300	Yellow-bellied terrapin	13,000	2,800	475
300	Yellow bellied ter. (heated)	10.000	60,000	∞
300	Heated serum +0.2 normal turtle serum	1,600	1,100	600
300	Bouillon +0.2 normal turtle serum	1,700	850	8,000
300	Human serum	3,300	25,000	∞
300	Human serum +0.2 turtle serum	2,100	1,600	20,000
300	Heated human serum +0.2 turtle serum	1.800	1,000	8,000

Sources of streptococci: No. 244 from blood, during life, in puerperal sepsis; No. 270 from blood, during life, in scarlet fever; No. 300 from heart's blood of scarlet-fever patient, post-mortem; No. B104 from an abscess in a guinea pig which had been injected with a fungus.

Several attempts were made to reactivate heated turtle serum with other sera, but these attempts all failed. Attempts to activate human serum with small quantities of turtle serum gave no better results than when bouillon was used instead of human serum. In summing up, we can say that streptococci multiply rapidly in the blood sera of most cold-blooded animals. Turtle serum has considerable bacteriolytic power over these organisms, and it would seem that this property is connected with the presence of a special complement.